

Applicant : Su-May Yu et al.
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REMARKS

Applicants hereby submit that the enclosures fulfill the requirements under 37 C.F.R. §1.821-1.825. The amendments in the specification merely insert the paper copy of the Sequence Listing and sequence identifiers in the specification. No new matter has been added.

Attached hereto is a marked-up version of the changes made to the specification by the current amendment.

Please apply any charges or credits to Deposit Account No. 06-1050, referencing attorney docket no. 08919-067001.

Respectfully submitted,

Date: 4-19-2002

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"Version With Markings to Show Changes Made"

In the specification:

Paragraph beginning at page 14, line 27, has been amended as follows:

The 1351-bp glutelin gene promoter region was PCR-amplified using rice genomic DNA as template and B1-5 (5'-GGGGAATTCGATCTCGATTTTTGAGGAAT-3' (SEQ ID NO:3), EcoRI site underlined) as forward primer and B1-3 (5'-GGGGGATCCCATAGCTATTTGTAC TTGCT-3' (SEQ ID NO:4), BamHI site underlined) as reverse primer. The glutelin gene promoter plus 75-bp putative signal peptide sequence was PCR-amplified using rice genomic DNA as template and B1-5 as forward primer and B1-sp (5'-GGGGGATCCGGGATTAAATAG CTGGGCCA-3' (SEQ ID NO:5), BamHI site underlined) as reverse primer. The truncated Apu encoding amino acid 106 to 1060 was PCR-amplified using genomic DNA of *T. ethanolicus* 39E as template and oligonucleotides 5'-CGGGATTCTTAAGCTTGCATCTTGA-3' (SEQ ID NO:6) (BamHI site underlined) as forward primer and 5'-CCGGCGGCCGCCTACATATTTTC CCCTTGCCA-3' (SEQ ID NO:7) (NotI site underlined) as reverse primer.

Paragraph beginning at page 15, line 9, has been amended as follows:

The PCR-amplified GluB-1 promoter and GluB-1 promoter-signal peptide sequence were digested with EcoRI and BamHI and subcloned into the same sites in pBluescript (Stratagene) to generate pBS-G and pBS-Gp. The truncated Apu was digested with BamHI and NotI and fused downstream of the GluB-1 promoter and GluB-1 promoter-signal peptide sequence in pBS-G and pBS-Gp, respectively, to make translational fusion and to generate pBS-G-Apu and pBS-Gp-Apu. The nopaline synthase gene terminator (Nos 3') was PCR-amplified using pBI221 (Clontech) as DNA template and oligonucleotide 5'-TCCGAGCTCCAGATCGTTCAAA CATTT-3' (SEQ ID NO:8) (SacI site underlined) as forward primer and oligonucleotide 5'-AGCGAGCTCGATCGATCTAGTAACAT-3' (SEQ ID NO:9) (SacI underlined) site as reverse primer. The Nos 3'UTR was digested with SacI and fused downstream of Apu in pBS-G-Apu and pBS-Gp-Apu to generate pBS-G-Apu-Nos and pBS-Gp-apu-Nos.

Paragraph beginning at page 15, line 20, has been amended as follows:

The 1.2 kb promoter and signal peptide sequence of α Amy8 was excised with SalI and HindIII from pAG8 (Chan et al., 1993, *supra*) and subcloned into pBluescript to generate pBS/8sp. The α Amy8 3'UTRs was PCR-amplified using RAMYG6a as DNA template and oligonucleotide 5'-CGCCGCGGTAGCTTTAGCTATAGCGAT-3' (SEQ ID NO:10) (SacII site underlined) as forward primer and oligonucleotide 5'-TCCCCGCGGGTCCTCTAAGTGAA CCGT-3' (SEQ ID NO:11) (SacII underlined) site as reverse primer. Plasmid RAMYG6a contains the 3' half portion of coding sequence and 3' flanking region of α Amy8 genomic DNA and was generated by screening of a rice genomic DNA library (Clontech) using α Amy8-C as a probe (Yu et al. (1992) Gene 122: 247-253). The α Amy8 3'UTRs was subcloned into the SacII sites in pBS/8sp to generate pBS/8sp8U. The truncated apu was cut with BamHI and NotI and subcloned into the same sites in pBS-8sp8U to generate pBS- α Amy8-sp-Apu-8U.

Paragraph beginning at page 16, line 24, has been amended as follows:

The truncated Apu encoding amino acids 106 to 1060 was PCR-amplified using genomic DNA of *T. ethanolicus* 39E as template and oligonucleotides 5'-CGCATATGTTAAGCTTGCA TCTTGATTC -3' (SEQ ID NO:12) as forward primer and 5'-CCGCTCGAGCTACATATTTT CCCCTTGGCCA-3' (SEQ ID NO:13) as reverse primer. The amplified DNA fragment was digested with NdeI and XhoI and ligated into the same sites in pET20b(+) (Novagen) to generate pET-APU. pET-APU was transferred to *E. coli* strain BL21 (DE3) and APU was expressed. Purification of APU was performed according to the instruction provided by Novagen. One hundred micrograms of purified APU was injected into a New Zealand White rabbit successively at 4-6 week interval according to the methods described by Williams et al. (1995, Expression of foreign proteins in *E. coli* using plasmid vectors and purification of specific polyclonal antibodies, in: DNA Cloning 2-Expression Systems-A Practical approach. (Ed) Glover and Hames, IRL Press, New York).